Handling the Image files in Iplab.

1) Once the chips have been scanned then they are saved onto the linux server witchfire under the appropriate folder name. Because the network connection between Iplab and witchfire is flacky you are advised to move all the images you want to process that day to your desktop. If your desktop is full then the images should be moved to your temporary directory in Quincy. (Quincy is accessed through Witchfire on the DIR_SCL domain)
2) Start Iplab arraysuite 1.3
3) File – Open as. Find your first image. Since these images are tiffs make sure the "Tiff" option is selected in the open file dialog box
4) Select "File" – "Open as" and then find your second image
5) Select the Cy3 (532nm) image
6) Select Enhance – Rotate and Scale. Confirm the angle is 90 and the nearest neighbor method is selected and press OK
7) Select Enhance – Flip. Confirm Flip horizontal is chosen and press OK
8) Select Enhance – Adjust Contrast. Change the contrast as seen fit (between 4 and 16 is usually ok) but make sure to remember number in the max box. Press OK
9) Use the paintbrush in the status window to mark the four corners of every subarray. There should be a total of 16 marks for images with 4 subarrays and 32 marks for images with 8 subarrays. You will have to estimate where the lower left mark of every subarray is located since our arrays are not perfectly square.
10) File – Save as. Make sure the file is going to the appropriate directory on Witchfire's Inbox. Make sure the Iplab format is selected in the Save dialog box. The new image file should use the following naming convention pXXsXX_532_flpmrk.ip
11) Now select the Cy5 (635nm) image.
12) Rotate the image as in step 6
13) Flip the image as in step 7
14) Adjust the contrast of the image using the same number you did in step 8
15) File – Save as. Use the naming convention pXXsXX_635_flp.ip

Using the Dearray Extension

1) Ext – DeArray.
2) The treated image should be selected for sample 1 (top)
3) The control image should be selected for sample 2 (bottom)
4) The scanner format should be NIH 2xM for 2x2 arrays and NIH(SKN) for 2x4 arrays.
5) Select the gipo for your print from the chips ahoy directory in Quincy

6) Verify the following before pressing go. Target size 10, Threshold weight 3, Local background subtraction, Intensity average within target, confidence 99.00%, Intensity range from 1 to 65535, and min target 0.

7) Visually inspect the processing result image. You want to make sure that all grids were aligned properly and that the larger prints such as the 12k were not clipped on the right edge.

Using the Target Locator Extension

1) Ext – Target Locator.
2) Press the "S" key once to balance the color image (balanced 1).
3) Press the "E" key to enhance the color image(enhanced).
4) Press the "R" key and then the "G" key to visualize the location of the outliers. If the red and green boxes are evenly distributed thought the entire array then the Hybe distribution is Uniform in the QAQC sheet. If the distribution seems skewed (i.e one side has noticeably more greens than the other) then choose Patchy.
5) Press the "F" key and change the search type to Clone ID. Type in xxxx and find the values for the blanks.   Make a rough estimate of the average of these blanks. Write down this number in the notes section of the QAQC form. Also write down the intensity of the brightest spot on the max blank intensity part of the QAQC form.
6) Press "R" then "G" so that the boxes disappear. Now take a snapshot of this color image (Open Apple + Shift +4). It will be saved as picture_1 on your harddrive.
7) Press "D" to enter the dialog window
8) Click the scatter button and then the log scale button.
9) For the human 12K and the Toxchip make sure the calibration is performed by selecting the internal controls button. For mouse, rat, yeast, and xenopus chips the calibration will be done by using all targets. Make note of your calibration choice on the QAQC sheet.
10) Take another snapshot (picture2)
11) Click on the histogram button followed by the target size button. Adjust your target size to a number between 30-100 by looking at the histogram. All you are trying to do is filter out the small broken spots. There is no need to go above 100.
12) Now click on the red channel button and look at the overall intensity distribution, then click on the green channel button and compare the distribution of the signal intensities of both channels. You are looking to see if intensity distribution of both channels is similar.
13) Click on the equal intensities button and use the search criteria to determine how many spots have intensities about 15000 for the green channel and then red channel. Combine what you learned with the histograms in step 10 with what you learned with this search and use the QAQC sheet to circle the criteria for the Cy3/Cy5 Distribution.

14) Click on the scatter plot button and type in the number you found for the average intensities of the blanks in step 5. This number is an indicator of what your intensity cutoff should be. It is also a good idea to play around with this number to eliminate the hook while at the same time trying to keep the cutoff as low as possible. A good method is to start with the average blank intensity and then increase your cutoff in small increments until the hook is gone.

15) Click the calibrated result button.

16) Take a snapshot of the new scatter plot (picture_3).

17) Take a snapshot of the calibrated ratio histogram (picture_4).

18) Take a snapshot of the red channel histogram (picture_5).

19) Take a snapshot of the green channel histogram (picture_6).

20) Take a snapshot of the target size histogram (picture_7).

21) Write down the CV and M value on the QAQC sheet.

22) Write down the intensity from, intensity to, and target size values on the QAQC sheet as well.

23) Click the update datasheet button followed by the ratio outliers button. Record the total number of outliers generated at 99.0% along with the ratio upper limit (RUL) and ratio lower limit (RLL).

24) Save the outlier list to Quincy/For Download to Maps/Outlier Purgatory.

25) Repeat steps 23 and 24 for the 90.0%, 95%, 99.5% and 99.9% confidence level. It should be noted that you must be viewing the scatterplot in order for the RUL and RLL to change.

26) On the QA/QC sheet one should mark whether this chip should be omitted from validation along with the digital storage location.

27) Press the exit button and double click on the color image to exit target locator.

28) Save the color image to the inbox using the format pXXsXX_color.tif

29) Save the sample intensity file to the inbox as pXXsXX_si.txt

30) Go to your hardrive and find your 7 screen shots. Rename the file by placing the word "picture" with the appropriate slide number by creating a file with the following format (pXXsXX_1)

31) Copy the 7 screenshots to Quincy/(Paules or Afshari)/From (your name).

32) Put the completed QAQC sheet in either Cindy or Rick's.

TargetLocator (v1.3) Key Code

<	Image Zoom Out
>	Image Zoom In
C	Control Genes Boundary Toggle
B	All Target Boundary Toggle
L	Array Layout Toggle
R	Red Target Toggle
G	Green Target Toggle
S	Background Subtraction Toggle
E	Enhance Image Toggle
D	Data Analysis Dialog Box
F	Find Gene
V	View in Array or Microtiter Plate mode
Option key	Will toggle magnifier to zoom out on IPLab images
Shift key	When held down one can use mouse to move images